American Journal of Medical Genetics 65:218–221 (1996)

Evidence for a Critical Region for Penoscrotal Inversion, Hypospadias, and Imperforate Anus Within Chromosomal Region 13q32.2q34

O. Bartsch, U. Kuhnle, L.L. Wu, E. Schwinger, and G.K. Hinkel

Institute of Clinical Genetics, University Hospital Carl Gustav Carus, Technical University Dresden (O.B., G.K.H.) and Institute of Human Genetics, Medical University of Lübeck (E.S.), Germany; and Department of Pediatrics, University Kebangsaan Malaysia, Kuala Lumpur (U.K., L.L.W.), Malaysia

Two unrelated patients with small distal deletions of the long arm of chromosome 13 are described, with shawl scrotum and penoscrotal transposition, penoscrotal hypospadias, a reduced perineum, and anal atresia. The patients have small deletions of 13(q32.2qter) and 13(q32q34), respectively. This report and the literature present evidence for one or possibly more gene(s) within region 13q32.2q34 which regulate the development of the ano-genital structures. The clinical spectrum includes bifid or shawl scrotum, hypospadias, biseptate uterus, malplaced and imperforate anus, and common cloaca. © 1996 Wiley-Liss, Inc.

KEY WORDS: chromosome 13, partial monosomy 13q, chromosomal bands 13q32 and 13q33 and 13q34, anal atresia, hypospadias

INTRODUCTION

Partial deletion of the long arm of one chromosome 13 was first described by Lele et al. [1963] in a patient with mental retardation and retinoblastoma. Subsequently, more than 100 cases have been reported, including some with unbalanced translocations, interstitial deletions, and ring chromosomes. The phenotype is variable. A 13q- syndrome of microcephaly with high nasal bridge, eye defect and thumb hypoplasia was delineated by Allderdice et al. [1969]. Additional attempts have been made to correlate the extent of the deletion with the phenotype [Niebuhr, 1977; Tranebjaerg et al., 1988]. Brown et al. [1993] preliminarily defined a "critical region" for malformations in 13q32 and proposed that the more distal deletions result in mental retardation without gross malformation. We describe here two unrelated patients with malformations of the ano-genital region and deletions of 13(q32.2qter) and 13(q32q34), respectively.

Received for publication June 19, 1995; revision received October 25, 1995.

Address reprint requests to Dr. Oliver Bartsch, Institute of Clinical Genetics, University Hospital, Fetscherstr. 74, D-01307 Dresden, Germany.

CLINICAL REPORTS

Patient 1

Patient 1 is the first child of a 25-year-old G1P0 and a nonconsanguineous father. The parents were healthy Malaysians. Antenatal history was unremarkable. He was born at term via C-section because of breech presentation in December 1992. Birth weight was 2,250 g. He had a short neck, apparently low-set ears and was hairy and relatively pigmented, in particular around the areolae and genitalia. The major abnormality was noted in the anogenital region (Fig. 1a,b). There was a penoscrotal transposition with hypospadias and opening of the urethra at the base of the phallus. Normal gonads were palpated in the bifid scrotum. The anus was anteriorly placed and there was a low type imperforate anus. Anoplasty was performed on the third day of life. The operation and the postoperative course were uneventful. A voiding cysturethrogram (MCU) at 6 weeks showed grade III bilateral vesico-ureteric reflux. At 18 months old, he weighed 7 kg, length was 73.9 cm (below −2 S.D.), head circumference (OFC) 42 cm (below −2 S.D.). The mouth was held open and the palate was highly arched. Psychomotor development was retarded. He could walk holding on, and he would say mama only, but he would understand simple commands. At age 23 months he was admitted for surgery of his genital abnormalities. He collapsed and arrested and was admitted to the Intensive Care Unit, where he died 2 days later. Apparently he had had recurrent upper respiratory tract infections during the last 3 months which were accompanied by fever. Of note was an enlarged cardiac size seen on X-ray. Echocardiography demonstrated a large ostium secundum with dilated right atrium, right ventricle and pulmonary artery, and good left ventricular function. Autopsy was denied by the parents.

Cytogenetic and fluorescent in situ hybridization (FISH) studies. Chromosome analysis by GTG banding documented a karyotype of 45,XY,−13,−22,+der

Fig. 1. **A:** Full view of patient 1 at age 23 months. Note hairy forehead, tented upper lip, and abdominal scar after neonatal anoplasty. **B:** Genital view of patient 1 at age 23 months, showing penoscrotal transposition, micropenis and malplaced anus. **C:** Genital view of patient 2 at age 7 months. Note shawl scrotum and reduced perineum. Both patients had penoscrotal hypospadias and anal atresia.

(13;22) (Fig. 2a). Parental chromosomes were normal. Breakpoints were studied by FISH and two different DNA probes. p22–94 is an alphoid DNA probe for chromosomes 22 and 14 [Jorgensen et al., 1988] and was used with the protocol of Bartsch and Schwinger [1991] and minor modifications. p22–94 indicated the presence of a full size no. 22 centromere on the der(13;22). Thus, the der(13;22) was a dicentric chromosome. The secondary constriction on the dic(13;22) was located at the 13 centromere which hence was the active centromere. The no. 22 centromere was inactive. P5426 (Oncor, Gaithersburg, MD) is a single copy DNA probe for the q-telomeric region of chromosome 13 (locus D13S327) and was used according to the manufacturer's instructions. P5426 hybridized to the normal chromosome 13 but not to the abnormal no. 13. The combined cytogenetic and FISH results placed the chromosomal breakpoints within bands 13q32.2 and 22p11, respectively. By FISH the deletion includes D13S327.

Molecular DNA analysis. Molecular analysis of the patient and his parents was performed by polymerase chain reaction (PCR) using standard methods and polymorphic microsatellite markers of the telomeric region of chromosome 13 including D13S265, D13S159, D13S274, D13S173, D13S285 and D13S293 [Gyapay et al., 1994]. The loci are ordered corresponding to their relative chromosomal order; the first locus (D13S265) is the closest to the centromere of chromosome 13, and the last locus (D13S293) is the closest to the telomere of the long arm of chromosome 13. The determination of the deletion breakpoint was based on the presence or absence of parental alleles. PCR analysis showed two alleles for marker D13S265 and a deletion of the paternal allele at loci D13S274 and D13S285. Thus, the deletion breakpoint is located between D13S265 and D13S274, and maps at 51 cM maximum distance and 31 cM minimum distance from D13S293, which is the most distal marker on chromosome 13q [Gyapay et al., 1994]. D13S159, D13S173, and D13S293 were not informative and showed one allele only. This is consistent with the deletion at distal 13q but can as well represent homozygosity.

Patient 2

Patient 2 is the second child of a 35-year-old mother and a nonconsanguineous 44-year-old father, both healthy and from Germany. He was born vaginally at 36 weeks gestation in June 1988. Birth weight was 2,250 g (−1.0 S.D.), length 48 cm, OFC 33 cm, and Apgar 8/10. The pregnancy was uneventful. An older sister had died in 1981 two hours after birth with a large gastroschisis. Inspection showed a shawl scrotum, penoscrotal hypospadias, a markedly reduced perineum, and anal atresia (Fig. 1c). Surgery was carried out and demonstrated an ano-perineo-scrotal fistula and anal atresia of the translevatoric type. Development was retarded. At 7 months, weight and length were 8.1 kg (−0.5 S.D.) and 72 cm, respectively. He had microcephaly with an OFC of 41.5 cm (−2.5 S.D.), blepharophimosis, small nose, large ears with prominent antihelices, highly arched palate, short neck, and brachydactyly. He was severely hypotonic. Functional testing indicated a developmental age of 3 months (Munich Developmental Scale). Cranial CT scan showed frontal brain atrophy. Because of the location of the chromosomal deletion, factor VII and X levels were determined. They were reduced to 61% and 42%, respectively, consistent with heterozygosity for a deletion.

Chromosome analysis. Prenatal chromosomal findings after amniocentesis for maternal age seemed to be normal at a resolution of 250–300 bands. At age 7 months, renewed analysis of cultured blood lymphocytes by high resolution GTG banding (450 band level) disclosed the small interstitial deletion of 13q with band 13q33 deleted and breakpoints within bands q32 and q34 (Fig. 2b).

DISCUSSION

We have described 2 unrelated patients with malformations of the ano-genital region and small distal deletions of 13q. The association of imperforate anus, perineal fistula, hypospadias and bifid scrotum in male patients, or biseptate uterus in female patients, was reported previously in a number of patients with deletions of chromosome 13q [Niebuhr, 1977; Brown et al., 1993] but only in rare occasions in combination with other chromosome aberrations. Exceptional cases include a patient with apparent Opitz (BBBG) syndrome and ring chromosome 22 [Christodoulou et al., 1990] and a patient with the Currarino triad (anorectal malformation, sacral bone abnormality and presacral mass) and partial trisomy of chromosomes 13q and 20p [Nagai et al., 1994]. Therefore, it is reasonable to as-

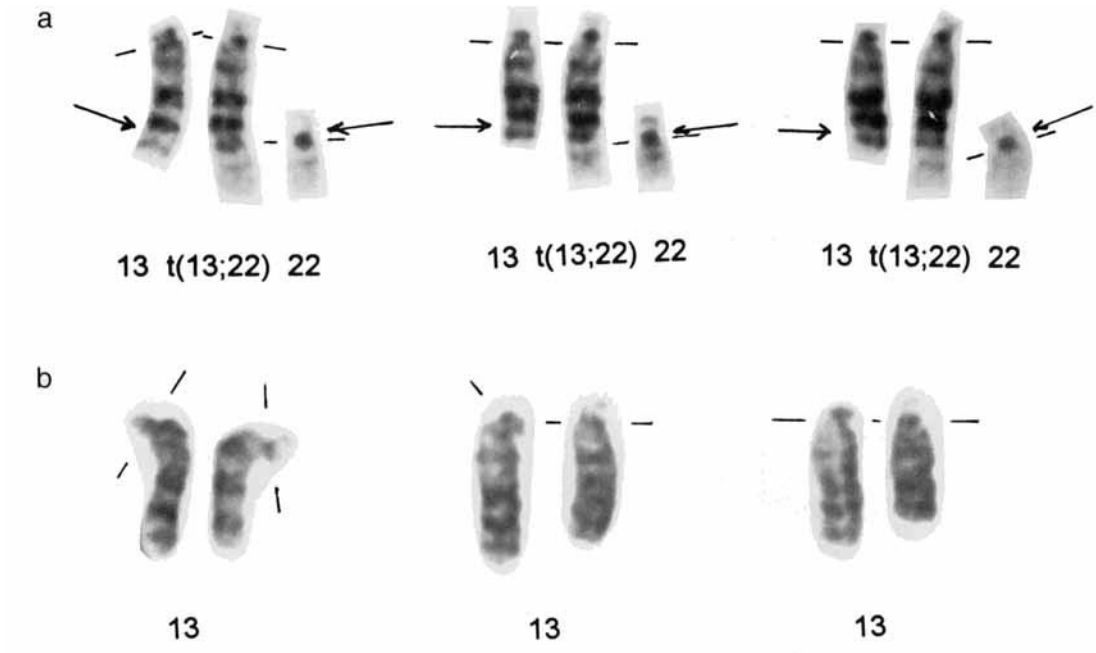

Fig. 2. Cytogenetic findings by G-banding. **a:** Case 1, normal chromosome 13 **(left),** dic(13;22)(q32.2p11; **middle)** with the Giemsa-dark band representing a no. 22 centromere, and normal no. 22 **(right)**. The arrows indicate the breakpoints. **b:** Case 2, normal no. 13 **(left)** and interstitial deletion 13(q32q34; **right)**.

sume that the ano-genital anomalies of patients 1 and 2 are related to and caused by the region of overlap of the chromosomal deletions. The critical region includes the distal portion of band 13q32, 13q33, and the proximal segment of 13q34.

Brown et al. [1993] reviewed 20 patients with 13q deletions and malformations, and defined a critical region for major malformations in 13q32. They proposed that the most distal deletions involving 13q33q34 are not associated with gross malformations. However, Brown et al. [1993] did not include ano-genital anomalies among those considered to be "major malformations" and associated with deletions of 13q32.

Four patients with deletion 13q and major ano-genital anomalies had been reported previously. Clinical manifestations included absent uterus [Carmichael et al., 1977], imperforate anus and ambiguous genitalia [Vittu et al., 1989], common cloaca, and imperforate anus, respectively [cases 12 and 13, Brown et al., 1993]. The deletion interval shared by these patients is 13q32q34. This is consistent with the findings in the present cases.

The association of terminal deletion 13q and mild ano-genital anomalies was reported more frequently. In the series of patients with ring chromosome and terminal deletion 13q reviewed by Niebuhr [1977], the incidence of imperforate anus was 8/41 (19%), biseptate uterus 2/5 (40%), hypospadias 8/20 (40%), and bifid scrotum 7/20 (35%). These and other observations [Pfeiffer et al., 1982; Brown et al., 1993] indicate that ano-genital abnormalities are frequent findings in the distal deletion of 13q.

In his detailed analysis of chromosome 13 deletions, Niebuhr [1977] reported that the abnormalities of the genitalia occur in both sexes and with similar frequencies. The defects involve the abdominal midline structures and include a common cloaca at the severe end of the clinical spectrum. The severity of the anal as well as the genital malformations is similar in affected patients [Carmichael et al., 1977; Vittu et al., 1989; Brown et al., 1993; this report]. This is consistent with a common regulatory defect involving one or more developmental gene(s). Homozygosity of the gene(s) would usually provide sufficient gene product for normal development, whereas loss of homozygosity (LOH) would result in ano-genital anomalies in those individuals where the remaining allele(s) have a reduced genetic activity (haploinsufficiency).

Further studies of sporadic patients with ano-genital anomalies using a panel of different DNA probes for chromosome 13q32.2q34 may help to elucidate the role of chromosomal microdeletions, and genes, in this region.

ACKNOWLEDGMENTS

We thank Kamarulzaman Othman for photography, Arleta Frensel for her excellent technical assistance in fluorescence in situ hybridization, Torsten Reichelt for contributing the molecular findings, and Walter Werner for help with cytogenetic evaluation. Ursula Kuhnle is the recipient of a grant from the German Academic Exchange Service.

REFERENCES

Allderdice PW, Davis JG, Miller OJ, Klinger HP, Warburton D, Miller DA, Allen FH, Abrams CAL, McGilvray E (1969): The 13-deletion syndrome. Am J Hum Genet 21:499–512.

Bartsch O, Schwinger E (1991): A simplified protocol for fluorescence in situ hybridization with repetitive DNA probes and its use in clinical cytogenetics. Clin Genet 40:47–56.

Brown S, Gersen S, Anyane-Yeboa K, Warburton D (1993): Preliminary definition of a "critical region" of chromosome 13 in q32: Report of 14 cases with 13q deletions and review of the literature. Am J Med Genet 45:52–59.

Carmichael A, Addy DP, Shortland-Webb WR, Cameron AH, Davies CS (1977): 13q- syndrome family study. Arch Dis Child 52: 972–974.

Christodoulou J, Bankier A, Loughnan P (1990): Ring chromosome 22 karyotype in a patient with Opitz (BBBG) syndrome. Am J Med Genet 37:422–424.

Gyapay G, Morisette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994): The 1993–94 Généthon human genetic linkage map. Nature Genet 7:246–339.

Jørgensen AL, Kolvraa S, Jones C, Bak AL (1988): A subfamily of alphoid repetitive DNA shared by the NOR-bearing human chromosomes 14 and 22. Genomics 3:100–109.

Lele KP, Penrose LS, Stallarf HB (1963): Chromosome deletion in a case of retinoblastoma. Ann Hum Genet 27:171–174.

Nagai T, Katoh R, Hasegawa T, Ohashi H, Fukushima Y (1994): Currarino triad (anorectal malformation, sacral bone abnormality and presacral mass) with partial trisomy of chromosomes 13q and 20p. Clin Genet 45:272–273.

Niebuhr E (1977): Partial trisomies and deletions of chromosome 13. In Yunis JJ (ed): "New Chromosomal Syndromes." New York: Academic Press, pp 273–299.

Pfeiffer RA, Ott R, Gilgenkrantz S, Alexandre P (1982): Deficiency of coagulation factors VII and X associated with deletion of a chromosome 13(q34). Evidence from two cases with 46,XY,t(13;Y)(q11;q34). Hum Genet 62:358–360.

Tranebjaerg L, Brøndum-Nielsen K, Tommerup N, Warburg M, Mikkelsen M (1988): Interstitial deletion 13q: Further delineation of the syndrome by clinical and high-resolution analysis of five patients. Am J Med Genet 29:739–753.

Vittu G, Croquette MF, Donney A, Duminy FR, Couturier J, Cousin J (1989): Syndrome polymalformatif léthal avec délétion 13q secondaire à une translocation maternelle X;13. J Génét Hum 37: 141–147.